Environmental Health Perspectives
Vol. 77, pp. 73-82, 1988

Interspecies Comparisons of Tissue DNA Damage, Repair, Fixation, and Replication

by Thomas J. Slaga*

The many anatomical, physiological, and biochemical differences among various mammalian species make it difficult to extrapolate carcinogenic potency data from animals to humans. The process is further complicated by the multistep origin of most malignant tumors in animals and humans due to the interaction of target cells with both endogenous and exogenous factors. Species differences in these aspects of carcinogenesis must also be considered when attempting to evaluate the carcinogenic risks of chemicals to humans. Cancer development in animals involves at least three distinct stages: initiation, promotion, and progression. Intra- and interspecies differences in susceptibility to carcinogenesis may be related to any one or a combination of these stages. Variation in species susceptibility to tumor initiation may result from differences in the abilities of various species to metabolize a potential carcinogen to an ultimate carcinogenic form and/or to detoxify the carcinogen. Most comparative studies among species have only revealed subtle differences in metabolism.

DNA adducts from several activated carcinogens have been found to be the same in a number of tissues from various species, including humans. Capacity for DNA repair is apparently a critical factor in the initiation of carcinogenesis in target cells of different species but is less critical among mice that differ in susceptibility to two-stage carcinogenesis of the skin and liver. Susceptibility variations among stocks and strains to such carcinogenesis appear to be related to alterations in tumor promotion. Additional comparative studies are critically needed on all aspects of carcinogenesis to permit effective extrapolation of carcinogenic potency data from animals to humans.

Introduction

Epidemiological data and studies with experimental animals have provided important clues that chemicals in our environment are responsible for a significant portion of human cancer (*1,2*). Although there are many natural and synthetic chemicals that induce cancer in experimental animals, it is very difficult to determine their possible activity in humans because no epidemiological data exist (*3–5*). The many anatomical, physiological, and biochemical differences among the various mammalian species make it very difficult to extrapolate carcinogenic potency data from experimental animals to humans. The development of cancer in humans appears to involve at least three distinct stages: initiation, promotion, and progression. Intra- and interspecies differences in susceptibility to carcinogenesis may be related to any one or a combination of these stages.

Although many species differences have been found in response to chemical carcinogens and/or tumor promoters, there have been few critical investigations of this subject. In most instances the studies were not designed for rigorous comparative analysis. Differences were often noted only as interesting sidelights of a study that did not focus primarily upon comparative aspects. Furthermore, for obvious reasons, human data are only obtained from epidemiological studies or from *in vitro* studies with human tissues or cells. Currently, very little is known about what determines carcinogenic potency, organ specificity, or species specificity. In some cases, differences in metabolic activation and/or detoxification, differential rates of repair of specific DNA adducts, tumor promotion, or cofactor interactions have been found and associated with organ and species specificity (*3–5*). The detailed comparative biochemistry of the mechanisms underlying these differences is poorly understood, however, and there should be extensive investigations in order to eliminate this gap in our knowledge.

It is not the intent of this paper to present an exhaustive review on the comparison of tissue damage by carcinogens in various species and the subsequent repair, fixation, and replication processes, but only to highlight the known critical events and factors that appear to be associated with species differences in multistage chemical carcinogenesis.

Species Differences in Carcinogenesis

A wide variety of chemicals are known to induce malignant tumors in experimental animals, especially in

*The University of Texas System, Cancer Center, Science Park-Research Division, P.O. Box 389, Smithville, TX 78746

rodents, and to a lesser degree in nonhuman primates. The focus of this paper will be to describe the effects of two classes of chemicals, polycylic aromatic hydrocarbons (PAHs) and nitrosamines. Many compounds in these classes, as well as many other carcinogens, are considered indirect carcinogens because they must be metabolically activated (*1*). Inasmuch as metabolic activation of chemical carcinogens appears to be one of the important factors in species differences, especially related to quantitative differences but not necessarily to qualitative differences, attention will be given to this category of chemicals (*5*).

Direct-acting carcinogens are also an important category of chemicals that are highly reactive. Insofar as carcinogens in this category have been tested, they show fewer species differences (*5*). Carcinogenicity and organ specificity of direct-acting carcinogens are highly dependent on the route of administration and are usually active at the site of application (*6*).

Table 1 summarizes the response of several species to carcinogenic PAHs, nitrosamines and nitrosamides, aromatic amines, and mycotoxins. As indicated in Table 1, each of these agents is responsible for some form of tumor development with little qualitative difference between species. Although there have only been a few comparative studies with mycotoxins, there also appears to be little qualitative difference in tumor response for the species that have been studied; however, these studies have shown quantitative differences in tumor response, the organs affected, specific characteristics of the carcinogen, and the route of administration (*3,5*).

In terms of PAH carcinogens, species differences were observed when PAHs were given either SC or topically (*3*). As shown in Table 2, the rat and fowl were found to respond positively to subcutaneous carcinogenesis but poorly to skin carcinogenesis, whereas the rabbit showed the opposite response (*3*). The mouse is very sensitive to tumor induction, the hamster is only moderately responsive to either route, and the guinea pig is poorly responsive to either route of administration (*3*). Benzo[a]pyrene (BP), dibenz[a,h]anthracene (DBA), and 3-methylcholanthrene (MC) were found to be negative in Old and New World monkeys (*7*), but MC and BP were active in more primitive primates (*8*). Although pure PAHs have not been directly related to human cancer, there is suggestive evidence that petroleum tars, mineral oils, and waxes are active (*3*). As shown in Table 3, the various stocks and strains of mice also have different responses to skin carcinogenesis by PAH but to a lesser degree than among the various species listed in Table 2 (*9*).

Although they induce tumors of different cell types and in different organs among species, as a group the nitrosamines are carcinogenic in all species examined (*10,11*). This variability in organ specificity for nitrosamines is probably due to species differences in routes of metabolism and activation (*10*). Table 4 summarizes the various species differences and organ specificity for nitrosamines. Both dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) are potent liver carcinogens in all species examined (Table 4). More than likely, these compounds are also liver carcinogens in man, considering that in all species examined they are metabolized in a similar manner, give the same adducts, and lead to similar hepatotoxicity (*10–12*). Furthermore, many different types of nitrosamines (e.g., symmetrical and asymmetrical nitrosodialkylamines and cyclic nitrosamines) have been found to be carcinogenic in a number of different species (*10*).

The route of administration appears to be an important consideration for nitrosamine toxicity. In general, the nitrosamines can be considered systemic carcino-

Table 1. The response of different species to various classes of carinogens.[a]

Species	Carcinogen			
	Poycyclic aromatic hydrocarbons	Nitrosamines and nitrosamides	Aromatic amines	Mycotoxins (aflatoxin B_1)
Mouse	+	+	+	+
Rat	+	+	+	+
Hamster	+	+	+	
Guinea pig	+	+	+	
Rabbit	+	+	+	
Dog	+	+	+	
Monkey	+/−	+	+/−	+
Man	+/−	+/−	+/−	+/−

[a] (+) Represents a carcinogenic response by at least one member of a class of compounds in some tissue of a given species; (+/−) refers to a borderline effect or insufficient data. Refer to Tables 2 and 4 for differences in carcinogens from a given class and for organ differences.

Table 2. The response of different species to the carcinogenic action of polycyclic aromatic hydrocarbons (*3*).

Species	Route of administration	
	Skin painting	SC injection
Mouse	+ + + + +	+ + + + +
Rabbit	+ + + +	±
Rat	+ +	+ + + + + +
Guinea pig	+ +[a]	+ +[b]
Hamster	+ + +[a]	+ + +
Fowl	±	+ + + + +
Dog[c]	+ +	?±
Man[c,d]	+ + +	?±

[a] High proportion of melanomas.
[b] High proportion of liposarcomas.
[c] Very long latent period (7 years or more).
[d] Refers to carcinogenic tars, mineral oils, waxes, etc., as no information is available with reference to pure hydrocarbons.

Table 3. Sensitivity to skin carcinogenesis in different stocks and strains of mice (*9*).[a]

Complete carcinogenesis
Sencar > CD-1 > C57BL/6 ≥ BALB/c ≥ ICR/Ha Swiss > C3H
Two-stage carcinogenesis (initiation-promotion)
Sencar > CD-1 > ICR/Ha Swiss > BALB/c ≥ C57BL/6 ≥ C3H ≥ DBA/2

[a] Data represent sensitivities to benzo[a]pyrene and dimethylbenzanthracene. Ranking represents a subjective analysis because dose-response data were not available for many strains.

Table 4. The carcinogenicityof some representative nitrosamines in various species.[a]

Compound	Species	Liver	Kidney	Esophagus	Lung	Nasal sinuses	Stomach	Bladder	Brain	Skin
Dimethylnitrosamine (DMN)	Rat	+ + +	+		+	+ + (?)				
	Mouse	+ +	+		+ + +					
	Hamster	+ +								
	Guinea pig	+								
	Trout	+								
Diethylnitrosamine (DEN)	Rat	+ + +	+	+ +		+				
	Mouse	+ +		+	+	+	+			
	Hamster	+ +			+	+				
	Guinea pig	+ + +			+					
	Rabbit	+								
	Dog	+								
	Monkey	+								
	Trout	+								
Dibutylnitrosamine	Rat	+ + +		+ +				+ +		
	Mouse	+ +		+	±		+ +	+		
	Hamster				+ +	+	+ +	+ +		
Methylphenylnitrosamine (*N*-methyl-*N*-nitroso-aniline)	Rat			+ +						
	Mouse				+ +					
N-nitrosomorpholine	Rat	+ + +	+			+ +				
	Mouse	+ +		+ + +						
	Hamster			+						
N-nitrosopiperidine	Rat	+ + +		+ + +	±	+ +	+			
	Hamster				+					
	Mouse	+					+			
N,N'-dinitrosopiperazine	Rat	±		+ +		+ +				
	Mouse	+			+ + +					
N-methyl-*N*-nitrosourea	Rat		+ +		+ +		+ +	+ +	+ + +	
	Mouse		+ +							+
N-methyl-*N*-nitrosourethane	Rat		+	+	+ +		+ + +		+	
	Mouse				+ +		+ +			+
N-methyl-*N*-nitroso-*N'*nitroguanidine	Rat						+ +			
	Mouse	+			+		+ +			+ +
	Dog						+ + +			

[a] Modified from Berenblum (*3*).

gens and not local carcinogens. However, those nitrosamides that are direct-acting carcinogens are carcinogenic at the site of application (*6*).

There are a number of reasons that the nitrosamines have become a matter of great concern in relation to human carcinogenesis. The nitrosamines are quite prevalent in the human environment and can develop spontaneously in the stomach by the interaction of two noncarcinogenic substances, secondary amines and nitrites. All species that have been tested are responsive to the carcinogenic action of these compounds, strongly suggesting that they are carcinogenic to humans, and, under certain experimental conditions, a single dose can be carcinogenic.

Several other classes of carcinogens such as aromatic amines, hydrazines, and mycotoxins are carcinogenic in several species of animals and may thus be carcinogenic in humans. The liver and bladder are the most common targets for a number of different aromatic amines in various species (*3,13*). Epidemiological data also suggest that certain aromatic amines are carcinogenic in humans (*1,13*). Although different hydrazines have been found to be carcinogenic in rats and mice, there have not been many species comparison studies performed with other species (*3,5,14*). Mycotoxins, such as aflatoxin B_1, are potent carcinogens in a number of different species, including monkeys (*7,15*). Epidemiological data, as well as data from several species showing similarity of metabolism and adduct formation, suggest that aflatoxin B_1 may be carcinogenic in humans (*15*).

Metabolic Activation of Chemical Carcinogens to Electrophilic Intermediates and DNA Binding

Miller and Miller (*1*) have proposed a general unifying theory to explain the initial event in chemical carcinogenesis. They state that all chemical carcinogens that are not electrophilic reactants must be converted metabolically into chemically reactive electrophilic forms which then react with some critical nucleophilic site(s)

in macromolecules to initiate the carcinogenesis. As there have been extensive species comparison studies on the metabolic activation and DNA binding of many types of chemical carcinogens, this discussion will focus on the metabolic activation and DNA binding of PAHs and nitrosamines.

There is considerable evidence supporting the existence of many species-specific, organ-specific, and cell-specific isozymic forms of the xenobiotic-transforming enzymes responsible for metabolizing PAH carcinogens (*16,17*). The carcinogenic PAHs are metabolized by the microsomal mixed-function oxidase system of target tissues to a variety of metabolites such as phenols, quinones, epoxides, dihydrodiols, and dihydrodiol-epoxides (*17,18*). As depicted in Figure 1, the major pathway of activation of BP leads to the formation of a dihydrodiol-epoxide of BP, which interacts predominantly with the 2-amino of guanine of DNA (*18,19*). The dihydrodiol-epoxide of BP appears to be the major ultimate mutagenic and carcinogenic metabolite of BP (*18,20,21*). Nevertheless, one should keep in mind that other metabolites, such as certain phenols, epoxides, and quinones, may contribute to the overall carcinogenic activity of BP (*21*). In addition, a free radical mechanism may also be partly involved in its carcinogenic activity (*22*).

In the case of carcinogenic PAHs, it appears that one of the major determinants of carcinogenic potency relates to the substrate specificity of the cytochrome P-450 mixed-function oxidase system (*23*). The extent to which a given parent PAH will be metabolized by the P-450 system to a reactive dihydrodiol-epoxide may be very critical with respect to carcinogenic potency. It appears that benzo[e]pyrene is noncarcinogenic or weakly carcinogenic because it is a poor P-450 system substrate for activation to a dihydrodiol-epoxide or any other reactive intermediate (*18*). Another important determinant of carcinogenic potency is related to the ability of various drug-metabolizing enzymes to detoxify the carcinogen (*16,17*). The presence, as well as the activities of metabolic activating and detoxifying enzymes, are important in carcinogen potency, organ specificity, and species differences (*16–18*).

Table 5 compares the metabolism of BP by cultured tracheobronchial tissue from various species (*24*). As

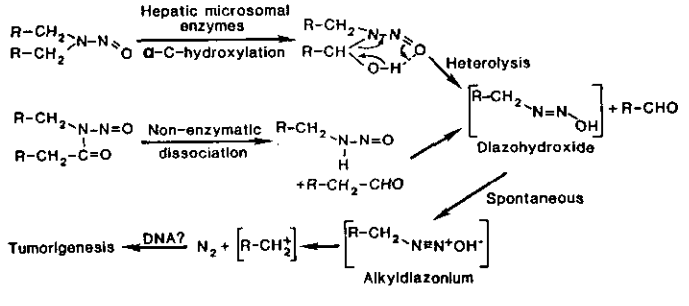

FIGURE 1. The major metabolic activation pathway of benzo[a]pyrene (BP) leading to its tumorigenic effect.

Table 5. Metabolism of benzo[a]pyrene (BP) by cultured tracheobronchial tissues.[a]

Species	Relative BP binding to DNA, %	Relative BP metabolism, %	Ratio, organo- / water-soluble metabolites
Human	100[b]	100[c]	0.4
Rat			
CD	41	10	0.2
Buffalo	47	10	0.2
Wistar	69	10	0.2
Mouse			
C57BL/6N	34	16	1.4
DBA/2N	28	6	0.5
Hamster	81	21	1.2

[a] Modified from Autrup et al. (*24*).
[b] The human BP binding data (BP bound per 10 mg DNA) were set at 100%, and the binding data from other species are relative to the human data.
[c] The human BP metabolism data (BP metabolized per 10 mg DNA) were set at 100%, and the metabolism data from the other species are relative to the human data.

can be seen, human tracheobronchial tissue is more effective than that of the rat, mouse, and hamster in the amount of BP metabolized, as well as in metabolizing BP to specific reactive intermediates that bind to DNA. Although there are quantitative differences in the various BP metabolites formed (e.g., phenols, quinones, dihydrodiols, and epoxides) in any given tissue or cell from various species, there are very few qualitative differences (*17*). Similarly, there is very little difference even in terms of specific BP adducts to target tissue DNA of various species, including humans (*19,24*). If overall metabolism of BP and binding of BP to DNA are indications of carcinogenic activity, then humans would be expected to be more susceptible to PAHs than mice, rats, or hamsters.

A number of studies have demonstrated a good correlation between the carcinogenicity of several PAHs and their ability to bind covalently with target tissue DNA of any given species (*25,26*). However, extensive dose-response studies, as shown in Figure 2, comparing BP skin tumor initiating activity and DNA binding in mice, have revealed a difference in the saturation of the tumor response and DNA binding (*27*). These data suggest that to find a strong correlation between these parameters, specific subpopulations of cells in a given target tissue and/or specific genes such as oncogenes in DNA for binding of the carcinogenic PAH should be examined.

The nitrosamines and nitrosamides are another important class of chemicals, because a large number of these compounds have been found to be carcinogenic. Nitrosamines require metabolic activation by target tissue microsomal mixed-function oxidases (*10*). Unlike PAHs, nitrosamines require only the first activation step, resulting in the formation of an hydroxylated intermediate that is sufficiently unstable to decompose spontaneously and generate a reactive carbonium ion. Alkylation of intracellular target macromolecules such

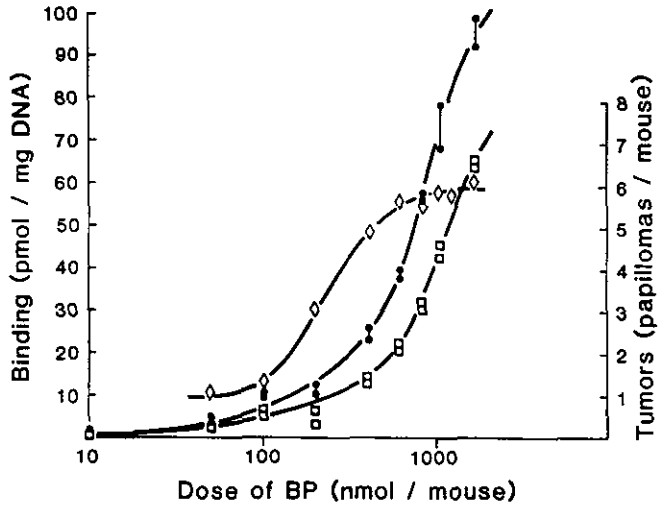

FIGURE 2. Semi-log plot of the dose-response relationships with benzo[a]pyrene (BP) for tumor initiation and covalent binding to DNA in mouse epidermis. For the tumor initiation experiments, BP was applied (at various doses to the skins of mice) as a single topical application. One week after initiation, mice received twice weekly applications of 3.4 nmole 12-O-tetradecanoylphorbol-13-acetate (TPA) for 24 weeks. For the binding experiments, [^{3}H]BP was applied at various doses and mice were sacrificed 24 hr later. The specific activity of [^{3}H]BP used for each dose was as follows: 10 nmole, 1.1×10^5 dpm/pmole; 50 nmole, 2.2×10^4 dpm/pmole; 100 nmole, 1.1×10^4 dpm/pmole; 200 nmole, 5.5×10^3 dpm/pmole; 400 nmole, 2.75×10^3 dpm/pmole; 600 nmole, 1.83×10^3 dpm/pmole; 800 nmole, 2.75×10^3 dpm/pmole; 1000 nmole, 2.2×10^3 dpm/pmole; and 1600 nmole, 1.38×10^3 dpm/pmole. Modified from Ashurst et al. (*27*). (◇) Average number of papillomas per mouse at 24 weeks of promotion; (●) total covalent binding of [^{3}H]BP metabolites to epidermal DNA expressed as pmole/mg DNA; (□) anti-BPDE bound to deoxyguanosine expressed as pmole/mg DNA.

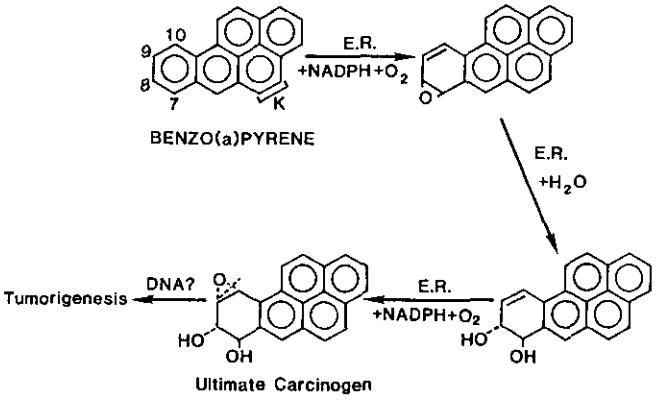

FIGURE 3. Metabolic pathway of activation of nitrosamines and nitrosamides that may be responsible for their tumorigenic effect.

as DNA or proteins follows rapidly (Fig. 3). Although the actual target site for nitrosamine carcinogenesis is not fully understood, a number of alkylation sites in DNA (e.g., O^6-methylguanine, 7-methylguanine, and 3-methyladenine) are known (*12*). In contrast, nitrosamides do not require metabolic activation because of their inherent chemical instability in aqueous solution (*6*). The nitrosamides are converted nonenzymatically at physiological pH to produce the same class of reactive electrophiles as those from the nitrosamines (Fig. 3).

N-Nitroso compounds are synthesized by reacting dietary secondary amines with nitrous acid under the naturally acidic conditions in the stomach (*28*). Nitrates in food preservatives and food color enhancers are converted by gastric juice to nitrosating compounds (*28,29*). Carcinogenic nitrosamines induce tumors in different organs in all species examined. The organ specificity of nitrosamines appears to be related to differences in metabolism and activation, but there is no clear understanding of this process. If one considers the nitrosamides, which show some organ specificity but do not have to be metabolized, the differences in susceptibility to carcinogenesis cannot be ascribed only to differences in metabolism (*6*). The rate of removal of specific adducts appears to be important in organ and species differences.

Investigations suggest that metabolic activation and DNA binding are important determinants in the carcinogenic activity of aromatic amines and mycotoxins in a number of different species. These adducts occur in most species, including humans, at the C-8 position of guanine (*1,14*). Aromatic amines possess reactive primary or secondary amine groups that are actively involved in determining their carcinogenicity (*1,13*). The metabolic activation of 2-acetylaminofluorine (AAF) involves N-hydroxylation (primarily at the C-8 position of guanine) followed by esterification forming a reactive carcinogen (*1,14*). In addition to interspecies differences in the ability to N-hydroxylate aromatic amines, there appear to be intraspecies differences as well (*1,30*). Takeishi et al. (*31*) have shown that *in vitro* guinea pig tissues are capable of N-hydroxylating AAF, but this is not seen in the intact animal. They suggested that the resistance of the intact guinea pig is related to the ability of this species to detoxify all of the activated metabolite (*31*). Experimental evidence indicates that aflatoxin B_1 (AFB_1) is metabolically activated to AFB_1-2,3-oxide, which is the ultimate reactive metabolite (*15*). The principal adduct of AFB_1 formed with DNA in all species examined is with the N-7 position of guanine (*15*). Booth et al. (*32*) compared the binding of AFB_1 to liver DNA from humans, rats, mice, and hamsters. They found that the binding level followed the same order as the potency of AFB_1 to induce liver cancer in these species (i.e., rat > hamster > mouse). Inasmuch as the binding level of AFB_1 to liver DNA from humans was found to be between that of the hamster and mouse, it is suggested that humans are much less sensitive than rats and are somewhat less sensitive than hamsters.

Table 6 summarizes the reaction with DNA of some chemical carcinogens discussed in this paper and selected examples of other carcinogens. There are many different types and sites of adduct formation with DNA for these several classes of carcinogens. Evidence suggests that certain adducts such as O^6-methylguanine are

Table 6. Summary of reactions of some chemical carcinogens or their metabolites with nucleic acids.[a]

Carcinogenic agents	Reactive intermediates	Sites of reaction in nucleic acids
2-Acetylaminofluorene	N-Hydroxylation followed by esterification	C-8 of guanine; N-2 of guanine
Aflatoxin B_1	2,3-Oxide of aflatoxins	N-7 of guanine; N-1, N-3 of adenine
Benzo[a]pyrene	Dihydrodiol epoxide	N-2 of guanine; exocyclic amine groups of adenine and cytosine
cis-Diamminedichloroplatinum (II)	Direct acting	Cross-links from O-6 of guanine to N-7 of guanine
Safrole	Ester of 1′-hydroxysafrole and/or 1′-hydroxy-2′,3′ oxide of safrole	O-6 of guanine
Mustards		
Difunctional	Direct acting	Inter- and intrastrand cross-links in DNA; DNA-protein cross-links
Monofunctional	Direct acting	
β-Propiolactone	Direct acting	N-7, N-3, N-1 of adenine
Alkyl sulfonates	Direct acting	N-7, N-3, N-1, O-6 of guanine
Alkyl nitrosamides	Direct acting (alkali catalyzed)	N-1, N-3, O-2 of cytosine N-3, O-4 of thymine
Alkyl nitrosamidines	Direct acting (alkali or thiol catalyzed)	Phosphate esterification
Dimethylnitrosamine	Oxidative demethylation: methyldiazonium hydroxide	

[a] Modified from Friedberg (*33*).

important in carcinogenesis and mutagenesis, but there is currently no definitive proof that any adduct(s) is obligatory in the carcinogenesis process. There is, however, a reasonable degree of correlation between carcinogenicity and the extent of carcinogen binding to DNA (*1,25,26*).

It is difficult to formulate a unified theory relating specific types of DNA damage to the mechanism of carcinogenesis, because adducts can react at multiple sites or may form multiple types of adducts. There is evidence that both major and minor DNA adducts formed by activated derivatives of BP, AAF, AFB_1, and certain nitrosamines are the same in different target tissues of various species, including humans (*1,19,22*). Table 7 lists several animal carcinogens that bind to DNA of various human tissues and in most cases form the same adducts (*24*). These data strongly suggest that these compounds are human carcinogens; however, there is variability in the binding data obtained from different human subjects (*24,34*) and it should be emphasized that some known carcinogens do not interact to a detectable extent with DNA (*19,35*).

DNA Repair, Fixation, and Replication

The capability for DNA repair is an important determinant of whether or not a cell becomes initiated. Since many chemical carcinogens or their reactive metabolites cause DNA damage, an important determinant of whether a cell becomes initiated by a carcinogen is DNA repair. Inhibition of the excision repair system or a faulty repair system enhances the chance that the carcinogenic damage will not be repaired, thus leading to the irreversible, initiated carcinogenic state (*36–38*). Inhibition of repair or induction of error-prone DNA could also lead to a carcinogenic process (*36,39*). The ability to repair DNA damage caused by both physical and chemical carcinogens could have profound effects on the susceptibility of various species to the induction of cancer by a given carcinogen. Cleaver and co-workers reported that human and monkey cells have a greater rate of excision repair than mouse cells (*20*). This finding suggests that mouse cells should be more susceptible to chemical carcinogens than human and monkey cells, based on several lines of evidence, including: ease of *in vitro transformation of mouse cells, difficulty of onco-*

Table 7. Chemical carcinogens activated to form DNA adducts by cultured human tissue.[a]

Carcinogen	*Bronchus*	*Colon*	*Esophagus*	*Pancreatic duct*	*Bladder*
Benzo[a]pyrene	+	+	+	+	+
7,12-Dimethylbenz[a]anthracene	+	+	+	+	
Nitrosodimethylamine	+	+	+	+	
Nitrosodiethylamine	+	+	+		
Nitrosopyrrolidine	+	+	−		
Aflatoxin B_1	+	+	+		+
1,2-Dimethylhydrazine	+	+	+		
2-Acetylaminoflourene	+		+		+

[a] (+) Carcinogen-DNA adducts were detected; (−) adducts not detected; no symbol, not tested. Modified from Autrup et al. (*24*).

genic transformation of human cells in culture, and greater cytotoxicity of mouse cells than human cells (*4,41*).

The importance of DNA repair in carcinogenesis is also suggested by the increased, if not invariable, incidence of cancer in people with hereditary DNA-repair defects, such as *xeroderma pigmentosum*, Fanconi's anemia, Bloom's syndrome, retinoblastoma, ataxia telangiectasia, and porokeratosis Mibelli (*40,42*). These hypersensitivity diseases are associated with various DNA repair defects and/or replication abnormalities, e.g., a person with xeroderma pigmentosum has an inherited hypersensitivity to ultraviolet light which can lead to skin cancer.

Three types of repairable DNA damage are known: (a) missing, incorrect, or altered DNA bases; (b) interstrand cross-links; and (c) strand breaks (*37*). DNA repair in mammalian cells can occur by at least two different processes, excision or postreplication repair (*36,37*). The excision repair process has been extensively studied in relation to carcinogenesis. Table 8 summarizes repair by the removal of carcinogen-DNA adducts by DNA repair enzymes (*37*). Currently, very little is known about the enzymatic mechanisms by which BP-, AAF-, and aflatoxin-DNA adducts are removed. Furthermore, little is known about the consequences that persistent adducts (i.e., those that are not repaired) have on DNA replication and gene transcription in mammalian cells from various species (*36,37*).

The mutagenic and carcinogenic effects of nitrosamines and nitrosamides have been associated with the formation and persistence in DNA of O^6-alkylguanine adducts (*12,43,44*). Persistent O^4-alkylthymine has been implicated as a mutagenic and carcinogenic lesion (*37,43*). Other DNA adducts, like 3-alkyladenine and 7-alkylguanine, are rapidly removed by specific *N*-glycosylases (*37*). The relative persistence of O^6-alkylguanine and possibly O^4-alkylthymine adducts appears to be related to organ and species differences in nitrosamine and nitrosamide carcinogenesis (*43*). The nitrosamines and nitrosamides are highly suspected to cause human cancer because the same adducts are formed and appear to persist in cultured human tissue (*24*).

As previously emphasized, there is a fairly good correlation in any given species between the amount of carcinogen bound to DNA and the tumor response (*1,19,26*). Furthermore, for any individual stock or strain of mouse, it has been generally observed that there is an excellent correlation between the amount of PAH bound to DNA and the skin tumor response (*26*). However, this correlation between DNA binding and tumor response breaks down when a comparison is made between mouse strains or stocks that differ in their tumor response to two-stage or to complete carcinogenesis (*9*). Phillips et al. (*45*) have demonstrated that the kinetics of binding of DMBA to the DNAs of C57BL/6, DBA/2, or Swiss mice were virtually identical in terms of formation and removal of adducts with time (Table 9). Although there is the possibility that a specific metabolite of the DMBA was responsible for the tumor response and was undetected in this study, recent investigations suggest that the major metabolites of DMBA and BP are qualitatively similar in mouse strains that vary in their response to two-stage or complete carcinogenesis with PAHs (*46*). The kinetics of DMBA binding to, and removal from, epidermal DNA in SENCAR and CD-1 mice are also similar to the C57BL/6, DBA/2, and Swiss strains of mice (*9,46*). Although these data are far from conclusive, they suggest that some aspects of initiation are probabaly similar in strains and stocks of mice that differ in their response to two-stage or complete carcinogenesis.

Drinkwater and Ginsler (*47*) also reported that the kinetics of DEN binding to liver DNA in two different strains of mice were very similar, even though their susceptibilities to DEN carcinogenicity were quite different. For example, when newborn male C57BL/6 and C3H mice were injected with DEN (0.1 μmole/g body weight) the mean liver tumor incidences at 32 weeks were 0.33 and 35 tumors per animal, respectively (*47*). This suggests that the difference in susceptibility to liver cancer by DEN is related to an event or events after initiation, such as excision and repair.

Table 8. Removal of carcinogen-DNA adducts by DNA repair enzymes.[a]

Product	Enzyme	Adduct
Oligonucleotide, nucleotide	Endonuclease/ exonuclease	Thymine dimer, probably aromatic amine, polycyclic hydrocarbon, etc.
Base	DNA glycosylase/ exonuclease	3-Methyladenine 7-Methylguanine Uracil Hypoxanthine
Adduct itself (O^6-methyl guanine)	*Adaptive enzyme,* methyl transferase	*Methyl group*

[a] Modified from Magee (*37*).

Importance of Tumor Promotion in Inter- and Intraspecies Differences

Most of the data that suggest the importance of tumor promotion in the inter- and intraspecies differences in carcinogenesis come from studies using skin as the target tissue. Furthermore, in terms of two-stage skin carcinogenesis, most of the data are based on studies that used a PAH as the initiator with croton oil or a phorbol ester as the promoter (*3,9*). Under these con-

Table 9. Binding of 7,12-dimethylbenz[a]anthracene to the DNA in the skin of mice of different strains.[a]

	DMBA bound (pmole/mg DNA) in mouse skin			
Strains	12 hr	24 hr	96 hr	192 hr
C57BL/6	22.0	52.4	19.2	8.3
DBA/2	30.5	41.0	19.0	10.5
Swiss	21.5	40.2	18.2	9.8

[a] Modified from Phillips et al. (*45*).

ditions, mice are much more sensitive than hamsters and rats (*3*). It is possible that, if a different initiator and/or promoter were used, the sensitivity to two-stage carcinogenesis in various species could be quite different.

DiGiovanni et al. (*48*) have examined the sensitivity of SENCAR, DBA/2, and C57BL/6 mice to skin tumor promotion by TPA (12-*O*-tetradecanoylphorbol-13-acetate). Their results, as well as those of others (*46,49*), have shown that SENCAR mice are more sensitive to TPA promotion than DBA/2 mice if DMBA (dimethylbenzanthracene) is used as the initiator. However, DiGiovanni et al. (*48*) have also found that the DBA/2 mice are as sensitive to TPA promotion as SENCAR mice if a direct-acting carcinogen such as *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (MNNG) is used as the initiator. Using either DMBA or MNNG, the C57BL/6 mice were resistant to TPA promotion (*48*). In addition, DiGiovanni and co-workers (*48*) have also found that susceptibility to TPA promotion appears to be inherited as an autosomal dominant trait in crosses between C57BL/6 and DBA/2 mice. Furthermore, susceptibility to liver tumor induction in various mouse strains and hybrids also appears to be related to tumor promotion (*47,50*).

It should be emphasized that SENCAR mice are sensitive to complete carcinogenesis as well as to two-stage carcinogenesis (*9*), whereas C57BL/6 mice are very refractory to two-stage skin carcinogenesis by BP-TPA. Even high initiating doses of BP (1600 nmole) and high promoting doses of TPA (10 μg) are very ineffective in causing skin tumors in this strain; however, C57BL/6 mice do respond to BP by complete carcinogenesis (*49*). This unequal susceptibility to complete and two-stage carcinogenesis within a stock or strain of mice strongly suggests that the promotional stages of complete and two-stage carcinogenesis are dissimilar. In addition, differences in sensitivity to initiation and promotion between mice may be due to alterations in the promotional stage of two-stage carcinogenesis. Benzoyl peroxide is an effective promoter in C57BL/6 and SENCAR mice when using DMBA as the initiator. In the DMBA-benzoyl peroxide experiments, SENCAR and C57BL/6 animals have similar tumor responses (*49*).

In order to understand the differences in susceptibility to TPA promotion in SENCAR and C57BL/6 mice, Slaga and co-workers (*22*) examined a number of biochemical and morphological responses assumed to be markers for skin tumor promotion. Although after a single topical treatment with TPA to SENCAR and C57BL/6 mice there were some differences in hyperplasia, dark cells, and ornithine decarboxylase activity, the differences were not great enough to account for the difference in tumor response (*22*). Furthermore, the skin of both mice contains specific receptors for TPA (*22,51*). TPA may not be an effective promoter in C57BL/6 mice because this strain lacks the ability to induce a sustained hyperplasia after repetitive TPA treatment (*9*). Sisskin et al. (*52*) have shown that the hamster (a species that is refractory to two-stage carcinogenesis) responds to a single treatment of TPA but loses responsiveness to repetitive treatment. These latter observations suggest the presence of an adaptive metabolizing enzyme for TPA.

Conclusion

Many factors at the molecular level appear to be involved in the inter- and intraspecies differences, in addition to organ differences, in carcinogenesis. Figure 4 summarizes the sequence of major events in chemical carcinogenesis that can cause species and organ differences in response to a given carcinogen. The following factors appear to play a critical role in species and organ differences: the overall balance of metabolic activation and detoxification of a carcinogen, the level and persistence of DNA damage, repair processes, and tumor promotion.

Although the level of response of a given chemical carcinogen may be influenced by differences in metabolic activation and/or detoxification, most studies have only revealed subtle differences in metabolism. It is quite remarkable that the major DNA adducts from several activated carcinogens have been found to be the same in target tissues from various species, including man; however, little is known about the importance of DNA adducts that appear in smaller amounts. DNA repair processes play important roles in species and organ responses to chemical carcinogens. Although the

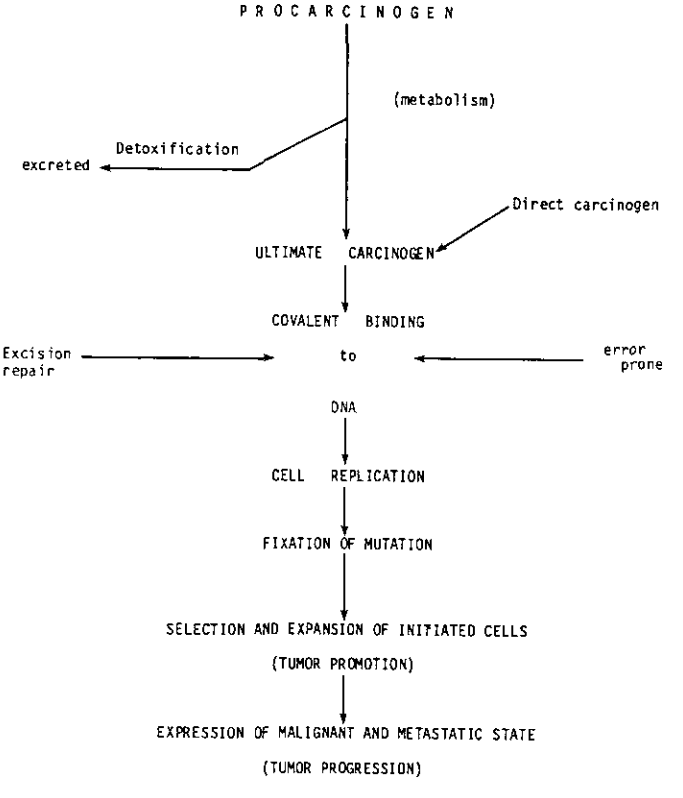

FIGURE 4. A schematic illustrating the major critical events in multistage carcinogenesis that are associated with organ and species differences.

results are inconclusive, it appears that persistent adducts from several classes of chemical carcinogens are critical in the induction of cancer in various species. The susceptibility to two-stage skin and liver carcinogenesis in stocks and strains of mice appears to be more closely related to tumor promotion than the interaction of the initiator with DNA or its removal. However, present understanding of the critical events in tumor promotion is incomplete.

Overall, if any chemical is found to be carcinogenic in at least one species and its binding to DNA or its removal from DNA is similar in target tissues of experimental animals and humans, then there is a high probability that the compound will be carcinogenic to humans; this is obviously not true for carcinogenic compounds that do not bind to DNA. In this case, if the chemical is carcinogenic in at least two species and if we do not have any consistent biochemical marker(s), then we must assume it would have a high probability of causing cancer in humans. Further comparative carcinogenesis studies are critically needed in order to effectively extrapolate carcinogenic potency data from experimental animals to humans.

REFERENCES

1. Miller, E. C., and Miller, J. A. The metabolism of chemical carcinogens to reactive electrophiles and their possible mechanisms of action in carcinogenesis. In: Chemical Carcinogens (C. E. Searle, Ed.), American Chemical Society, Washington, DC, 1976, pp. 737–762.
2. Slaga, T. J. Cancer: Etiology, mechanisms, and prevention—A summary. In: Modifiers of Chemical Carcinogenesis: An Approach to the Biochemical Mechanism and Cancer Prevention. Carcinogenesis: A Comprehensive Survey, Vol. 5 (T. J. Slaga, Ed.), Raven Press, New York, 1980, pp. 243–262.
3. Berenblum, I. Carcinogenesis as a Biological Problem. Frontiers of Biology, Vol. 34. American Elsevier, New York, 1974.
4. Harris, C. C., and Cerutti, P. A., Eds. Mechanisms of Chemical Carcinogenesis. Proceedings of the UCLA Symposium held at Keystone, CO, February 22–March 1, 1981. Alan R. Liss, New York, 1982, pp. 1–58.
5. Langenbach, R., Nesnow, S., and Rice, J. M., Eds. Organ and Species Specificity in Chemical Carcinogenesis. Plenum Press, New York, 1983.
6. Rice, J. M., and Perantoni, A. Organ specificity and interspecies differences in carcinogenesis by metabolism-independent alkylating agents. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 77–105.
7. Adamson, R. H., and Sieber, S. M. Chemical carcinogenesis studies in nonhuman primates. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 129–156.
8. Adamson, R. H., Cooper, R. W., and O'Gara, R. W. Carcinogen-induced tumors in primitive primates. J. Natl. Cancer Inst. 45: 555–559 (1970).
9. Slaga, T. J., and Fischer, S. M. Strain differences and solvent effects in mouse skin carcinogenesis experiments using carcinogens, tumor initiators and promoters. In: Progress in Experimental Tumor Research, Vol. 26 (F. Hamburger, Ed.), S. Karger, Basel, 1983, pp. 85–109.
10. Lijinsky, W. Species specificity in nitrosamine carcinogenesis. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 63–75.
11. Rice, J. M., and Frith, C. H. The nature of organ specificity in chemical carcinogenesis. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 1–22.
12. Montesano, R., Bresil, H., Planche-Martel, G., Pegg, A. E., and Margison, G. Modification of DNA repair processes induced by nitrosamines. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 531–543.
13. Weisburger, E. K. Species differences in response to aromatic amines. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 23–47.
14. Miller, E. C. Some current perspectives on chemical carcinogenesis in humans and experimental animals: Presidential address. Cancer Res. 38: 1479–1496 (1978).
15. Croy, R. G., Essigmann, J. M., and Wogan, G. N. Aflatoxin B_1: Correlations of patterns of metabolism and DNA modification with biological effects. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 49–62.
16. Juchau, M. R. Species and organ differences in biotransformation of chemical carcinogens. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 273–281.
17. Selkirk, J. K., MacLeod, M. C., Mansfield, B. K., Nikbakht, P. A., and Dearstone, K. C. Species heterogeneity in the metabolic processing of benzo[a]pyrene. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 283–294.
18. Jerina, D. M., Lehr, R. E., Yagi, H., Hernandez, O., Dansette, P. M., Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., and Conney, A. H. Mutagenicity of benzo[a]pyrene derivatives and the description of a quantum mechanical model which predicts the ease of carbonium ion formation from diol epoxides. In: In Vitro Metabolic Activation in Mutagenesis Testing. Proceedings of the Symposium on the Role of Metabolic Activation in Producing Mutagenic and Carcinogenic Environmental Chemicals, Research Triangle Park, NC, February 9–11, 1976 (F. J. de Serres, J. R. Fouts, J. R. Bend, and R. M. Philpot, Eds.), Elsevier, New York, 1976, pp. 159–177.
19. Weinstein, I. B. Current concepts and controversies in chemical carcinogenesis. J. Supramol. Struct. Cell Biochem. 17: 99–120 (1981).
20. Levin, W., Wood, A. W., Wislocki, P. G., Chang, R. L., Kapitulnik, J., Mah, H. D., Yagi, H., Jerina, D. M., and Conney, A. H. Mutagenicity and carcinogenicity of benzo[a]pyrene and benzo[a]pyrene derivatives. In: Polycyclic Hydrocarbons and Cancer, Vol. 1, Environment, Chemistry, and Metabolism (H. V. Gelboin and P. O.-P. Ts'O, Eds.), Academic Press, New York, 1978, pp. 189–204.
21. Slaga, T. J., Fischer, S. M., Triplett, L. L., and Nesnow, S. Comparison of complete carcinogenesis and tumor initiation and promotion in mouse skin: The induction of papillomas by tumor initiation-promotion—a reliable short term assay. J. Environ. Pathol. Toxicol. 4: 1025–1041 (1981).
22. Slaga, T. J., Fischer, S. M., Weeks, C. E., Klein-Szanto, A. J. P., and Reiners, J. Studies on the mechanisms involved in multistage carcinogenesis in mouse skin. J. Cell. Biochem. 18: 99–119 (1982).
23. Gelboin, H. V., and Ts'O, P. O.-P., Eds. Polycyclic Hydrocarbons and Cancer, Vols. 1 and 2. Academic Press, New York, 1978.
24. Autrup, H., Grafstrom, R. C., and Harris, C. C. Metabolism of chemical carcinogens by tracheobronchial tissues. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 473–495.
25. Brookes, P., and Lawley, P. D. Evidence for the binding of polynuclear aromatic hydrocarbons to the nucleic acids of mouse skin: Relation between carcinogenic power of hydrocarbons and their binding to deoxyribonucleic acid. Nature 202: 781–784 (1964).
26. Slaga, T. J., Buty, S. G., Thompson, S., Bracken, W. M., and Viaje, A. A kinetic study on the *in vitro* covalent binding of

polycyclic hydrocarbons to nucleic acids using epidermal homogenates as the activating system. Cancer Res. 37: 3126–3131 (1977).
27. Ashurst, S. W., Cohen, G. M., Nesnow, S., DiGiovanni, J., and Slaga, T. J. Formation of benzo(a)pyrene/DNA adducts and their relationship to tumor initiation in mouse epidermis. Cancer Res. 43: 1024–1029 (1983).
28. Mirvish, S. S., Wallcave, L., Eagen, M., and Shubik, P. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. Science 177: 65–68 (1972).
29. Montesano, R. Alkylation of DNA and tissue specificity in nitrosamine carcinogenesis. J. Supramol. Struct. Cell. Biochem. 17: 259–273 (1981).
30. Miller, E. C., Miller, J. A., and Enomoto, M. The comparative carcinogenicities of 2-acetylaminofluorene and its N-hydroxy metabolite in mice, hamsters, and guinea pigs. Cancer Res. 24: 2018–2031 (1964).
31. Takeishi, K., Okuno-Kaneda, S., and Seno, T. Mutagenic activation of 2-acetylaminofluorene by guinea-pig liver homogenates: Essential involvement of cytochrome P-450 mixed-function oxidases. Mutat. Res. 62: 425–437 (1979).
32. Booth, S. C., Bosenberg, H., Garner, R. C., Hertzog, P. J., and Norpoth, K. The activation of aflatoxin B_1 in liver slices and in bacterial mutagenicity assays using livers from different species including man. Carcinogenesis 2: 1063–1068 (1981).
33. Friedberg, E. C. DNA Repair. W. H. Freeman and Company, New York, 1985, pp. 38–39.
34. Harris, C. C., Autrup, H., Connor, R., Barrett, L. A., McDowell, E. M., and Trump, B. F. Interindividual variation in binding of benzo[a]pyrene to DNA in cultured human bronchi. Science 194: 1067–1069 (1976).
35. Farber, E. Cellular biochemistry of the stepwise development of cancer with chemicals: G. H. A. Clowes Memorial Lecture. Cancer Res. 44: 5463–5474 (1984).
36. Hanawalt, P. C., Cooper, P. K., Ganesan, A. K., and Smith, C. A. DNA repair in bacteria and mammalian cells. Annu. Rev. Biochem. 48: 783–836 (1979).
37. Magee, P. N. Introduction: DNA damage and repair. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 497–508.
38. Setlow, R. B. Repair deficient human disorders and cancer. Nature 271: 713–717 (1978).
39. Friedberg, E. C., Bonura, T., Love, J. D., McMillan, S., Radany, E. H., and Schultz, R. A. The repair of DNA damage: Recent developments and new insights. J. Supramol. Struct. Cell Biochem. 16: 91–103 (1981).
40. Cleaver, J. E., Bodell, W. J., Gruenert, D. C., Kapp, L. N., Kaufmann, W. K., Park, S. D., and Zelle, B. Repair and replication abnormalities in various human hypersensitive diseases. In: Mechanisms of Chemical Carcinogenesis. Proceedings of the UCLA Symposium held at Keystone, CO, February 22–March 1, 1981 (C. C. Harris and P. A. Cerutti, Eds.), Alan R. Liss, New York, 1982, pp. 409–418.
41. Borek, C., and Mohr, U. In vitro and in vivo carcinogenesis: Workshop report. In: Mechanisms of Chemical Carcinogenesis. Proceedings of the UCLA Symposium held at Keystone, CO, February 22–March 1, 1981 (C. C. Harris and P. A. Cerutti, Eds.), Alan R. Liss, New York, 1982, pp. 491–493.
42. Friedberg, E. C., Ehmann, U. K., and Williams, J. I. Human diseases associated with defective DNA repair. In: Advances in Radiation Biology, Vol. 8 (J. T. Lett and H. Adler, Eds.), Academic Press, New York, 1979, pp. 85–174.
43. Kleihues, P., Hodgson, R. M., Veit, C., Schweinsberg, F., and Wiessler, M. DNA modification and repair *in vivo*: Towards a biochemical basis of organ-specific carcinogenesis by methylating agents. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 509–529.
44. Pegg, A. E. Repair of alkylated DNA by cell extracts from various organs and species. In: Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, S. Nesnow, and J. M. Rice, Eds.), Plenum Press, New York, 1983, pp. 545–563.
45. Phillips, D. H., Grover, P. L., and Sims, P. The covalent binding of polycyclic hydrocarbons to DNA in the skin of mice of different strains. Int. J. Cancer 22: 487–494 (1978).
46. DiGiovanni, J., Slaga, T. J., and Boutwell, R. K. Comparison of the tumor-initiating activity of 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene in female SENCAR and CD-1 mice. Carcinogenesis 1: 381–389 (1980).
47. Drinkwater, N. R., and Ginsler, J. Genetic control of susceptibility to liver tumor induction in mice. Proc. Am. Assoc. Cancer Res. 25: 126 (1984).
48. DiGiovanni, J., Prichett, W. P., Decina, P. C., and Diamond, L. DBA/2 mice are as sensitive as SENCAR mice to skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate. Carcinogenesis 5: 1493–1498 (1984).
49. Reiners, J. J. Jr., Nesnow, S., and Slaga, T. J. Murine susceptibility to two-stage carcinogenesis is influenced by the agent used for promotion. Carcinogenesis 5: 301–307 (1984).
50. Diwan, B. A., Rice, J. M., Ohshima, M., and Ward, J. M. Interstrain differences in susceptibility to liver carcinogenesis initiated by N-nitrosodiethylamine and its promotion by phenobarbital in C57BL/6NCr, C3H/HeNCr$^{MTV^-}$, and DBA/2NCr mice. Carcinogenesis 7: 215–220 (1986).
51. Blumberg, P. M., Dunn, J. A., Jaken, S., Jeng, A. Y., Leach, K. L., Sharkey, N. A., and Yeh, E. Specific receptors for phorbol ester tumor promoters and their involvement in biological responses. In: Mechanisms of Tumor Promotion, Vol. 3, Tumor Promotion and Carcinogenesis In Vitro (T. J. Slaga, Ed.), CRC Press, Boca Raton, FL, 1984, pp. 143–184.
52. Sisskin, E. E., Gray, T., and Barrett, J. C. Correlation between sensitivity to tumor promotion and sustained epidermal hyperplasia of mice and rats treated with 12-O-tetradecanoylphorbol-13-acetate. Carcinogenesis 3: 403–407 (1982).